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Mechanisms of induction of chromosomal aberrations by hydroquinone in V79 cells

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Hydroquinone occurs naturally in bacteria and plants and it is also manufactured for commercial use. Human exposure to this compound can occur by environmental, occupational, dietary and cigarette smoke exposure and from exposure to benzene, which can be metabolized to this compound. However, the main source of exposure to this compound is dietary, since hydroquinone is a naturally occurring compound in many foods. Hydroquinone can be metabolized to benzoquinones, which are potent haematotoxic, genotoxic and carcinogenic compounds that can also induce the formation of radical species, predisposing cells to oxidative damage. In order to clarify the involvement of radical species in the genotoxicity of hydroquinone, the induction of chromosomal aberrations in V79 cells was studied along with the assessment of the production of hydroxyl radicals at different pH values (6.0, 7.4 and 8.0), as well as the effect of antioxidant enzymes [catalase and superoxide dismutase (SOD)] on the clastogenic effect of hydroquinone. The results obtained indicate that the clastogenic activity of hydroquinone is dependent on the pH, suggesting that deprotonation is a fundamental step leading to DNA lesions under the experimental conditions used. The addition of S9 mix, SOD or SOD and catalase significantly decreased the clastogenic activity, suggesting the involvement of superoxide anion and hydrogen peroxide in the genotoxicity of hydroquinone. However, other species generated in the auto-oxidation process of hydroquinone, such as the semiquinone radical or the quinone, also seem to play a role in its genotoxicity, since the addition of antioxidant enzymes (catalase and SOD) or S9 mix do not lead to a complete abolition of the observed genotoxic activity. These results suggest the existence of at least two mechanisms associated with the genotoxic activity of this compound.

Introduction

Epidemiological evidence suggesting a relationship between environmental factors and the incidence of certain human cancers (Doll and Peto, 1981) has led to considerable interest in identifying factors in our environment which might play a role in the aetiology of cancer.

Hydroquinone occurs naturally in bacteria and plants and it is also manufactured for commercial use. It is used in cosmetics and photography as a black and white developer, among other industrial applications (Doepker *et al*., 2000). Human exposure to hydroquinone can occur by environmental, occupational, dietary and cigarette smoke exposure (Ong *et al*., 1994) and from exposure to benzene, which can be metabolized to hydroquinone (Snyder and Hedli, 1996; Bolton *et al*., 2000). However, the main source of exposure to this compound is dietary, since hydroquinone is a naturally occurring compound in various foods (Doepker *et al*., 2000). Hydroquinone can be metabolized to benzoquinones, which are potent haematotoxic, genotoxic and carcinogenic compounds (Snyder and Hedli, 1996; Bolton *et al*., 2000). Hydroquinone can also lead to an intracellular accumulation of radical species predisposing cells to oxidative damage (Rao and Snyder, 1995; Andreoli *et al*., 1999), which has been associated with clastogenic effects induced by this compound (Dobo and Eastmond, 1994).

Hydroquinone is able to induce significant DNA damage in several test systems. Exposure of Syrian hamster embryo cells to hydroquinone induces gene mutations, unscheduled DNA synthesis, chromosomal aberrations, sister chromatid exchanges and cell transformation *in vitro* (Tsutsui *et al*., 1997). Hydroquinone also induces micronuclei in mouse bone marrow polychromatic erythrocytes *in vivo* (Marrazzani *et al*., 1994) and in human lymphocytes *in vitro* (Yager *et al*., 1990; Vian *et al*., 1995) and induces DNA strand breaks in this cell system (Andreoli *et al*., 1999).

Hydroquinone induces cellular transformation of BALBc/3T3 cells and 12-*O*-tetradecanoylphorbol-13-acetate enhances that effect, suggesting that this compound could behave as an initiator (Joseph *et al*., 1998). Hydroquinone is also a carcinogen in long-term experiments (Whysner *et al*., 1995). It can also enhance the carcinogenic effect of other compounds, such as methyl-*N*-amylnitrosoamine and *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (Yamaguchi *et al*., 1989; Kawabe *et al*., 1994; Wada *et al*., 1998).

Hydroquinone can undergo an oxidative process giving rise to reactive oxygen species and also quinone and/or semiquinone metabolites that can interact with proteins involved in microtubule assembly, spindle formation (Dobo and Eastmond, 1994) and human topoisomerase II (Franz *et al*., 1996) and these are properties that are potentially associated with its clastogenic effects (Dobo and Eastmond, 1994).

Phenolic compounds are known to auto-oxidize in aqueous solutions at physiological pH giving rise to active oxygen species such as the superoxide anion (Rosin, 1984), suggesting that the mechanisms of DNA damage by hydroquinone could be mediated by the production of radical species. However, other compounds arising from the auto-oxidation of hydroquinone could also be involved in the genotoxic effects induced by this compound (e.g. quinones).

In order to clarify the involvement of radical species on the genotoxicity of hydroquinone we have studied the induction of chromosomal aberrations in V79 cells, together with the

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production of hydroxyl radicals at different pH values (6.0, 7.4 and 8.0) and the effect of antioxidant enzymes [catalase and superoxide dismutase (SOD)] on its clastogenic effect.

Materials and methods

Chemicals and culture media

Hydroquinone, hydrogen peroxide, SOD from human erythrocytes, catalase, newborn calf serum and Ham's F-10 medium were obtained from Sigma (St Louis, MO). EDTA, Tris, $FeCl_3$, dimethylsulfoxide (DMSO) and Giemsa dye were from Merck (Darmstadt, Germany). Thiobarbituric acid (TBA) and colchicine were from Fluka (Buchs, Switzerland). Fungibact solution (10 000 IU/ml penicillin, 10 000 µg/ml streptomycin sulphate and 25 µg/ml amphotericin B) was from Irvine Scientific.

Rat liver extracts

S9 was prepared as described in detail by Maron and Ames (1983). Briefly, male Wistar rats were injected with Aroclor 1254 (500 mg/kg) in corn oil. Five days later, the rats were killed by cervical elongation. The animals were fed *ad libitum* and 12 h before death the food was removed. Livers were excised, washed extensively in 0.154 M KCl, homogenized in a Potter–Elvehjem homogenizer and centrifuged at 9000 *g*. The supernatant was collected and stored at –80°C. S9 mix (10% v/v) was prepared according to Maron and Ames (1983).

Chromosomal aberration assay

The V79 cell line has been described elsewhere in detail (Wolfel *et al*., 1991). Cells were cultured in 5 ml of Ham's F-10 medium supplemented with 10% newborn calf serum and 1% Fungibact solution (Irvin Scientific) and incubated at 37°C under an atmosphere of 5% CO_2. Twenty-two hour cultures (~1 × 10^6 cells) were washed with Ham's F-10 medium reconstituted in 0.01 M phosphate buffer, pH 6.2, 7.4 or 8.0, and grown in 5 ml of this medium for 2 h in the presence of hydroquinone dissolved in water (20, 40, 60 and 60 µM). For positive controls, 1.5 µM mitomycin C (Sigma) was used. When metabolic activation was required, 500 µl of 10% (v/v) S9 mix and 4.5 ml of medium were used. Cyclophosphamide (42.9 µM) was used as a positive control in the experiments in the presence of S9 mix. After the treatment cells were washed with culture medium and grown for another 16 h. Colchicine was added at a final concentration of 0.56 µg/ml and cells were grown for a further 3 h. Cells were then harvested by trypsinization. After 2 min hypotonic treatment with 0.56% (w/v) KCl at 37°C, cells were fixed with methanol/acetic acid (3:1) and slides were prepared and stained with Giemsa (4% in 0.01 M phosphate buffer, pH 6.8) for 10 min. Two independent experiments were carried out and 100 metaphases were scored for each dose level treatment group in each experiment. Scoring of the different types of aberrations followed the criteria described by Rueff *et al*. (1993). The cytotoxicity of hydroquinone was assessed by calculating the mitotic indices (MI) (per cent metaphases in 2000 cells) and when the dose tested induced a decrease in the MI of >50%, when compared with the control, it was considered to be cytotoxic.

Induction of chromosomal aberrations in the presence of SOD and catalase

These experiments were performed as described in Materials and methods using 80 µM hydroquinone at pH 7.4. SOD and catalase were added to the cultures during the 2 h of the treatment period with the compound studied. The activities used were 74.6 U (Marklund)/flask SOD and 265 U/flask catalase, which correspond to the activities of SOD and catalase present in 500 µl of S9 mix. Experiments with catalase (265 U) and SOD (74.6 U) alone were also performed.

The determinations of SOD and catalase activities in S9 were carried out as described by Marklund and Marklund (1974) for SOD and by Wheeler *et al*. (1990) for catalase.

Detection of hydroxyl radicals

Hydroxyl radicals were measured by the deoxyribose assay according to Laughton *et al*. (1989) by incubating for 2 h at 37°C in 1.2 ml of a reaction mixture composed of 10 mM potassium phosphate buffer, pH 6.0, 7.4 or 8.0, 27 µM hydroquinone, 2.8 mM deoxyribose, 20 µM $FeCl_3$ and 100 µM EDTA. Hydrogen peroxide (1.42 mM) was used as a positive control for hydroxyl radical generation. Deoxyribose degradation by hydroxyl radicals was measured by the TBA method using 1 ml of trichloroacetic acid (2.8%) and 1 ml of TBA (1%) in 0.05 M NaOH. The mixture was incubated at 100°C for different periods of time (0–120 min), cooled and the absorbance measured at 532 nm. The incubation time of 15 min was chosen for further testing. For each assay six independent experiments were performed. Negative controls (iron and EDTA without hydroquinone and hydroquinone without iron and EDTA) were performed. The absorbance values of the controls without hydroquinone were subtracted in each experiment. Kinetic experiments were also carried out in order to evaluate the rate of formation of hydroxyl radical. In these experiments the conditions were as described before but the reaction was stopped at different times.

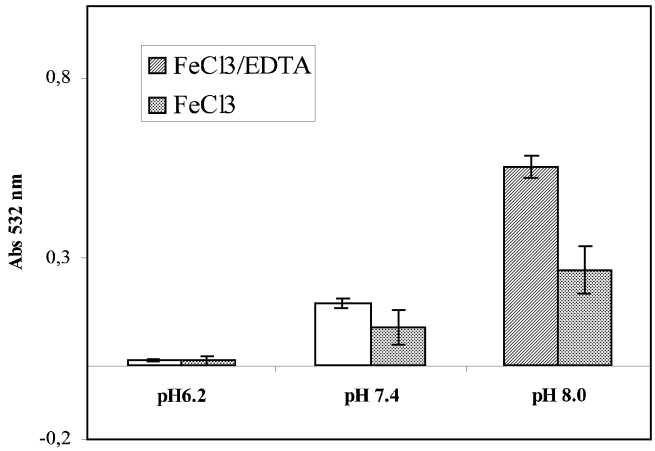

Fig. 1. Production of TBA-reactive products by hydroquinone in the presence of Fe^{3+}/EDTA or Fe^{3+} alone at different pH values.

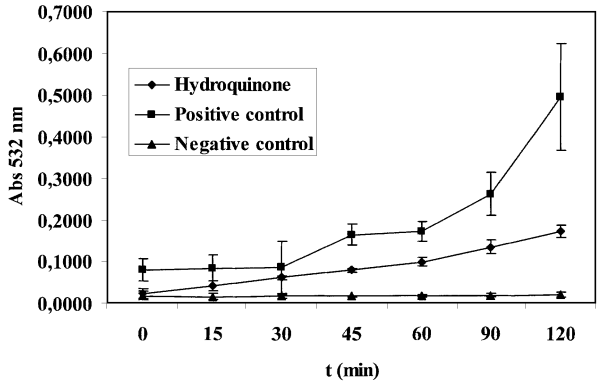

Fig. 2. Production of TBA-reactive products by hydroquinone at different times at pH 7.4 in the presence of Fe^{3+}/EDTA. These experiments were carried out in the presence of a positive control (1.42 mM hydrogen peroxide) and a negative control (reaction mixture in the absence of hydroquinone).

Statistical analysis

The statistical analyses of differences in chromosomal aberrations frequency in the different experiments were carried out using the *t*-test. All analyses were performed with an SPSS statistical package (version 10.5) (SPSS Inc., Chicago, IL).

Results

The ability of hydroquinone to generate hydroxyl radicals was measured by the deoxyribose assay, at different pH values in the presence of Fe^{3+}/EDTA or in the presence of Fe^{3+}. The results obtained showed that hydroquinone can generate $OH^{\cdot}$ radicals in a pH-dependent manner and that this effect is more pronounced in the presence of Fe^{3+}/EDTA than in the presence of Fe^{3+} alone (Figure 1). This observation is in agreement with the results published by Laughton *et al*. (1989), who showed that Fe^{3+}/EDTA is a more efficient generator of $OH^{\cdot}$ than Fe^{3+} alone in a Fenton-type reaction. Kinetic experiments showed that the production of hydroxyl radicals are clearly time dependent (Figure 2). This effect was also observed for the positive control (1.42 mM hydrogen peroxide) and was not observed in control experiments carried out in the absence of

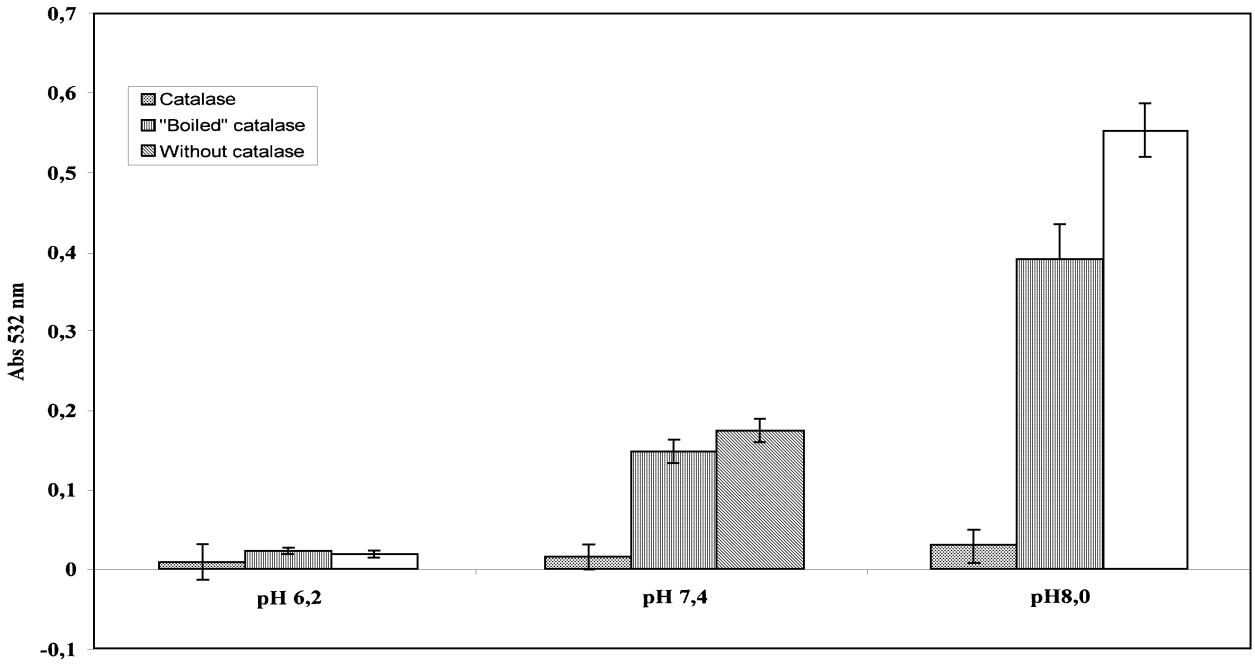

Fig. 3. Effect of catalase and heated catalase on the formation of TBA-reactive products by hydroquinone at pH 7.4 in the presence of Fe^{3+}/EDTA.

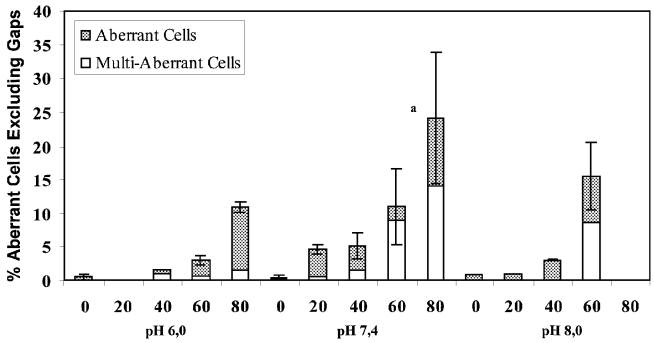

Fig. 4. Induction of aberrant and multi-aberrant cells by hydroquinone at different pH values. Values represent the average of at least two experiments, in which 100 cells per dose were scored. Error bars are the standard deviation of the mean. [a]$P < 0.05$ when the value is compared with the value of the negative control of the same pH.

any potential hydroxyl radical generator (hydroquinone or hydrogen peroxide). The production of hydroxyl radicals by hydroquinone seems to be mediated by the formation of hydrogen peroxide, since the addition of catalase to the reaction leads to an inhibition of hydroxyl radical production (Figure 3).

The results obtained indicate that the clastogenic activity of hydroquinone is dependent on the pH (Figure 4 and Table I). At pH 6.0 no significant increases in the level of chromosomal aberrations in V79 cells versus the negative control were observed for any of the doses studied (Figure 4 and Table I). At pH values of 7.4 and 8.0 hydroquinone induced a significant level of chromosomal aberrations at the higher dose studied (80 μM) (Figure 4 and Table I).

However, the increase in pH from 7.4 to 8.0 does not lead to a significant increase in the levels of chromosomal aberrations. This may be due to the rate of reactive oxygen species production being much higher at pH 8.0 (Figures 1 and 3) than at pH 7.4, leading to an increase in cytotoxicity as assessed by the decrease in MI (Table I). Additionally, we cannot rule out that at higher pH values the transport of reactive oxygen species formed by auto-oxidation in the medium, across the cell membrane, may be a limiting step of genotoxicity.

Considering the results with 80 μM hydroquinone at both pH 7.4 and 8.0, that dose was chosen for further testing.

Experiments carried out at pH 7.4 in the presence of S9 mix and hydroquinone (80 μM) showed that the presence of S9 mix significantly decreased ($P < 0.002$) the clastogenic activity of hydroquinone (Figure 5 and Tables II and III). This effect was mainly a consequence of the decrease in the level of multi-aberrant cells (Figure 5 and Tables II and III). Catalase did not have any significant effect on that activity, whereas SOD alone and SOD + catalase led to a significant decrease in the clastogenic activity ($P < 0.003$ and 0.002, respectively) (Figure 5 and Tables II and III).

Discussion

The results obtained on the production of $OH^{\cdot}$ by hydroquinone and its genotoxic activity under the experimental conditions used suggest that radical mechanisms, namely the production of $OH^{\cdot}$, could partially account for the genotoxicity of the compounds studied. The dependence of the genotoxicity on pH suggests that deprotonation is a fundamental step in the induction of DNA damage under our experimental conditions since a pH increase from 6.0 to 7.4 led to a significant increase in the induction of chromosomal aberrations.

Polyphenols, such as hydroquinone, can spontaneously oxidize at pH above neutrality, giving rise to the superoxide anion (Snyder and Hedli, 1996) and semiquinone intermediates:

$HQ \rightarrow HQ^{-} + H^{+}$

(HQ, hydroquinone; HQ^{-}, deprotonated hydroquinone)

$HQ^{-} + O_2 \rightarrow HQ^{-\cdot} + O_2^{-\cdot}$ ($HQ^{-\cdot}$, semiquinone)

Table I. Induction of chromosomal aberrations in V79 cells by hydroquinone at different pH values

pH	Hydroquinone (μM)	*n*	Chromosomal aberrations per 100 cells[a]							>10 (%)	CAEG/cell	ACEG (%)	MI (%)
			Ctg	Ctb	Csg	Csb	Dic	Rearr	Others				
6.0	0	5	0.6	0.6	0	0	0	0	0	0.2	0.01	0.6 ± 0.45	5.75 ± 1.90
	20	2	0.5	0	0	0	0	0	0	0.5	0	0.5 ± 0.71	
	40	2	0.5	0	0	1	0	0	0	0	0.01	1.0 ± 0.01	
	60	2	0	0	0	0	0.5	0	0	1.0	0.01	1.5 ± 1.42	
	80	2	0.5	0.5	0	0.5	0	0	0	0	0.01	1.0 ± 0.00	4.15 ± 0.21
	MMC (0.5 μg/ml)	2	1.0	4.5	0	1.0	0	2.0	0	7.0	0.08	14.0 ± 2.83	1.75 ± 0.92
7.4	0	15	0.3 (3)	0.1	0.1	0.1	0	0	0	0.2	0.00	0.3 (3) ± 0.41	7.08 ± 2.55
	20	2	1.0	0.5	0	0	0.5	0	0	0.5	0.01	1.5 ± 0.71	
	40	2	1.5	0	1.0	1.0	0.5	0.5	0	0	0.02	2.0 ± 0.01	
	60	2	0	0	1.0	0.5	0.5	0	0	2.0	0.01	3.0 ± 2.83	
	80	11	2.2	3.9	0.46	2.1	0.27	1.5	0.5	3.7	0.08	10.3 ± 4.13	3.73 ± 0.66
	MMC (0.5 μg/ml)	10	1.6	6.6	1.0	3.5	0.4	1.2	0.3	1.9	0.12	12.0 ± 1.79	4.56 ± 1.50
8.0	0	5	1.0	0.6	0	0.2	0	0	0	0	0.01	0.8 ± 0.01	10.10 ± 3.79
	20	2	0	0	0	1.0	0	0	0	0	0.01	1.0 ± 0.01	
	40	2	0	0	0	0	0.5	0	0	0	0.01	0.0 ± 0.01	
	60	2	1.5	0	0.5	0	0	0	0	0.5	0	0.5 ± 0.71	
	80	2	2.0	0	0	4.0	0.5	1.5	0	3.5	0.06	8.5 ± 4.95	3.85 ± 0.21
	MMC (0.5 μg/ml)	5	1.6	2.2	1.0	3.0	0.2	1.6	0	3.4	0.07	10.0 ± 3.78	3.80 ± 1.03

[a]These results are average values of at least two independent experiments (100 metaphases analysed per experiment).
n, number of independent experiments. Ctg, chromatid gap; Csg, chromosome gap; Ctb, chromatid break; Csb, chromosome break; Dic, dicentric chromosome; Rearr, rearrangements (tri-radial, quadri-radial and other complex rearrangements); CAEG/cell, chromosomal aberrations excluding gaps per cell, corresponding to the sum of Ctb, Csb, Dic, Rings and Rearr per cell; ACEG (%), per cent of aberrant cells excluding gaps (average ± SD); >10, multi-aberrant cells, corresponding to cells with >10 aberrations. These cells are included in the ACEG (%); MI, mitotic index.

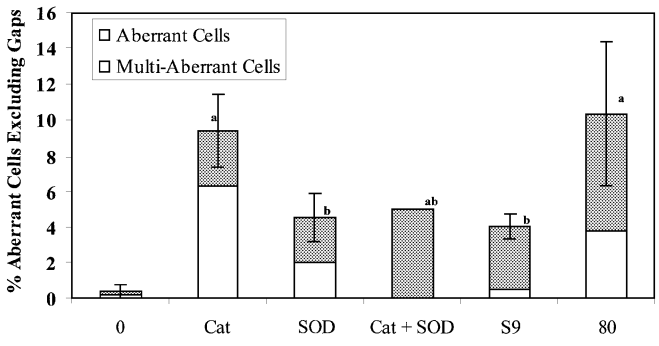

Fig. 5. Effect of S9 mix, SOD, catalase (CAT) and SOD + catalase on the induction of aberrant and multi-aberrant cells at pH 7.4. The values are the average of at least two experiments, in which 100 metaphases were scored. The error bars are the standard deviation of the mean. [a]$P < 0.05$ when the value is compared with the negative control. [b]$P < 0.05$ when the value is compared with the levels of chromosomal aberrations in the absence of any enzymatic system for the dose of 80 μM.

The superoxide anion produced in this way can then initiate a free radical chain reaction with concomitant production of other reactive oxygen species, such as hydrogen peroxide and hydroxyl radical. The ultimate reactive oxygen species towards DNA is considered to be the hydroxyl radical ($OH^{\cdot}$), generated from hydrogen peroxide by the Fenton reaction (Halliwell and Gutteridge, 1986). The genotoxicity of hydroquinone could partially be attributed to its auto-oxidation with concomitant production of radical species. Indeed, it has been demonstrated that superoxide anion/hydrogen peroxide generating systems are genotoxic to mammalian cells, namely to V79 cells (Tsuda, 1981; Dobo and Eastmond, 1994), and that quinones and semiquinones can also be genotoxic to mammalian cells (Dobo and Eastmond, 1994; Cadenas, 1995).

Since hydroquinone oxidizes extracellularly with production of superoxide anion its genotoxicity can be attributed to either hydrogen peroxide formed in the process, which can enter the cells and give rise to $OH^{\cdot}$ in the vicinity of DNA, or the protonated form of the superoxide anion ($HO_2^{\cdot}$), which is able to cross the cell membrane and give rise to hydrogen peroxide inside the cell with subsequent production of OH in the vicinity of DNA (Halliwell and Gutteridge, 1986; Rueff *et al*., 1992; Gaspar *et al*., 1994). However, other species generated in the auto-oxidation process of hydroquinone, such as the semi-quinone radical or the quinone, also seem to play a role in its genotoxicity, since the addition of antioxidant enzymes (catalase and SOD) or S9 mix do not lead to a complete abolition of the observed genotoxic activity.

Concerning the compound used as a positive control, mitomycin C, it was also observed that the presence of SOD led to a decrease in the genotoxicity of this compound (see Table II). Apart from bioreductive alkylation, which is the primary mechanism underlying the genotoxic effects of mitomycin C, an auto-oxidation mechanism that leads to the formation of superoxide anion has also been previously described, and this might explain the reduction in mitomycin C genotoxicity observed in the presence of SOD (Halliwell and Gutteridge, 1989).

Hydroquinone is clearly a clastogenic agent under our experimental conditions, which is in agreement with the results previously reported by Fabiani *et al*. (2001), Vian *et al*. (1995) and Yager *et al*. (1990) under similar experimental conditions, and its genotoxicity seems to be pH dependent. The ability to produce $OH^{\cdot}$ and to induce DNA breaking activity has also been reported for other compounds structurally related to hydroquinone, namely hydroxyhydroquinone (Hiramoto *et al*., 1998) and quercetin (Gaspar *et al*., 1994). The results obtained concerning the production of $OH^{\cdot}$ radicals, which is also pH dependent, suggests that the induction of chromosomal aber-

Table II. Induction of chromosomal aberrations in V79 cells by hydroquinone in the presence of S9 mix and antioxidant enzymes (SOD and catalase)

Hydroquinone (μM)	*n*	Chromosomal aberrations per 100 cells[a]							>10 (%)	CAEG/cell	ACEG (%)	MI (%)
		Ctg	Ctb	Csg	Csb	Dic	Rearr	Others				
0	15	0.3 (3)	0.1	0.1	0.7	0	0	0	0.2	0.00	0.3 ± 0.41	7.08 ± 2.55
80	11	2.2	3.9	0.46	2.1	0.27	1.5	0.5	3.7	0.08	10.3 ± 4.13	3.73 ± 0.66
MMC (0.5 μg/ml)	10	1.6	6.6	1.0	3.5	0.4	1.2	0.3	1.9	0.12	12.0 ± 1.79	4.56 ± 1.50
0 + S9 Mix	2	0	0.5	0	0	0	0	0	0	0.01	0.5 ± 0.01	7.15 ± 3.89
80+ S9 Mix	2	2.0	3.0	0	0	0	0	0.5	0.5	0.04	4.0 ± 0.71	7.35 ± 3.47
CP + S9 Mix	2	2.0	5.0	1.0	1.0	0.5	0	0.5	0.5	0.07	7.5 ± 0.71	8.85 ± 3.47
0 + SOD	3	0.667	0	0	0	0	0	0	0	0	0.0 ± 0.0	5.67 ± 1.25
80+ SOD	2	1.5	0.5	0	1.0	0	0	1.0	2.0	0	4.5 ± 1.41	3.30 ± 2.48
MMC + SOD	3	1.3 (3)	3.0	1.3 (3)	2.0	0	3.3 (3)	0	0.3 (3)	0.08	7.3 (3) ± 0.58	4.57 ± 3.52
0 + CAT + SOD	3	0	0	1.0	0	0	0	0	0	0	0.0 ± 0.0	4.83 ± 1.46
80 + CAT + SOD	3	2.0	3.3 (3)	1.0	0.3 (3)	0	0.7	0.33	0	0.05	5.0 ± 0.02	6.83 ± 2.16
MMC + CAT + SOD	3	1.0	2.0	1.0	1.0	0.3(3)	2.0	0.3(3)	0.3 (3)	0.06	5.67 ± 0.58	4.40 ± 0.78
0 + CAT	3	0	0	0	0	0	0	0	0	0	0.0 ± 0.0	5.70 ± 1.13
80 + CAT	3	0.7	2.7	0	0.3 (3)	0	0.7	0	6.3 (3)	0.04	9.3 (3) ± 2.12	2.53 ± 0.72
MMC + CAT	3	1.0	2.7	0.3 (3)	1.7	0	2.7	0.7	2.0	0.08	10.0 ± 1.73	3.73 ± 1.61

[a]These results are average values of at least two independent experiments (100 metaphases analysed per experiment).
n, number of independent experiments. Ctg, chromatid gap; Csg, chromosome gap; Ctb, chromatid break; Csb, chromosome break; Dic, dicentric chromosome; Rearr, rearrangements (tri-radial, quadri-radial and other complex rearrangements); CAEG/cell, chromosomal aberrations excluding gaps per cell, corresponding to the sum of Ctb, Csb, Dic, Rings and Rearr per cell; ACEG (%), per cent of aberrant cells excluding gaps (average ± SD); >10, multi-aberrant cells, corresponding to cells with >10 aberrations. These cells are included in the ACEG (%); MI, mitotic index.

Table III. Effect of catalase, SOD, SOD + catalase and S9 mix on the inhibition of the genotoxicity of hydroquinone considering the levels of chromosomal aberrations excluding gaps

	Inibition (%)			
	Catalase	SOD	SOD + catalase	S9 mix
Hydroquinone	9.5	56.2	51.3	61.1

rations by this compound may be partially mediated by $OH^{\cdot}$ radicals.

The involvement of oxygen radicals in the genotoxicity of hydroquinone was also indicated by previous studies, namely the induction of DNA damage in human lymphocytes, measured by the alkaline single cell gel electrophoresis technique, which is almost completely abolished when catalase is added to the reaction, suggesting that reactive oxygen species, but not metabolism, are involved in the induction of chromosomal aberrations (Andreoli *et al*., 1999). Exposure of HL60 cells to hydroquinone also leads to a significant increase in the levels of hydrogen peroxide (Rao and Snyder, 1995).

Besides chromosome breakage in human lymphocytes *in vitro*, hydroquinone was also shown to induce significant increases in the frequency of hyperploidy, although the majority of the lesions (67%) are due to chromosomal breakage (Rupa *et al*., 1997).

The results obtained in the present study also suggest that hydroquinone genotoxicity is dependent on the formation of the superoxide anion radical outside the cell, since only experiments conducted in the presence of SOD (S9 mix, SOD and SOD + catalase) led to a significant reduction in the clastogenic effects (39.7, 44.6 and 49.6%, respectively) (Table III). However, the clastogenic effects observed do not seem to be entirely accounted for by reactive oxygen species, since the addition of S9, SOD + catalase or SOD alone decreases the genotoxic effects observed but does not abolish the induction of chromosomal aberrations. The results suggest, indeed, the existence of at least two different mechanisms associated with the genotoxic activity of hydroquinone.

Since the reduction in clastogenic activity was about one-half in the presence of the enzymatic systems studied, it is possible to consider that, apart from reactive oxygen species, other compounds arising from hydroquinone auto-oxidation (e.g. quinones and semiquinones) could also be involved in the genotoxicity of this molecule. These results are in agreement with those involving the induction of CREST-positive and CREST-negative micronuclei in V79 cells. In these experiments CREST-negative micronuclei are significantly inhibited by the presence of catalase, although CREST-positive micronuclei are not inhibited, suggesting that quinone and/or semiquinone metabolites can interact with proteins involved in microtubule assembly, spindle formation (Dobo and Eastmond, 1994) or with human topoisomerase II (Franz *et al*., 1996), mechanisms which are potentially related to the aneugenic effects of hydroquinone. On the other hand, reactive oxygen species could contribute to chromosomal breakage induced by hydroquinone, being mainly associated with its clastogenic effects (Dobo and Eastmond, 1994). Benzoquinone, a well-known hydroquinone metabolite, can also react with DNA, giving rise to adducts (Chenna *et al*., 1995) that could also be involved in the mechanisms of genetic lesion induced by this compound.

The existence of several mechanisms of genotoxicity for this compound could also explain the synergistic effects observed between hydroquinone and other phenolic compounds in several genotoxicity test systems (Marrazzani *et al*., 1994; Chen and Eastmond, 1995) since phenolic compounds can share common mechanisms of genotoxicity. These results could also help to ascertain the differences observed in the levels and types of DNA lesion in several test systems, since the experimental conditions used (e.g. pH) or the different cellular levels of antioxidant enzymes or other enzymes associated with the metabolic fate of this compound [e.g.

SOD, glutathione *S*-transferase (Muñoz and Barnet, 2000) and NAD(P)H:quinone oxireductase 1 (Moran and Ross, 1999)] could lead to one preferential mechanism in the different test systems used.

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